09/619,047

attachment 1 E 21

(FILE 'HOME' ENTERED AT 14:52:02 ON 01 APR 2002)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 14:52:09 ON 01 APR 2002

L1	32172	S LUCIFERASE
L2	100	S L1 AND CASPASE
L3	65	DUP REM L2 (35 DUPLICATES REMOVED)
L4	35	S L3 AND CASPASE-3
L5	4	S L4 AND RENILLA

FILE 'STNGUIDE' ENTERED AT 14:53:55 ON 01 APR 2002

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 14:56:26 ON 01 APR 2002

L6	5	S L3 AND RENILLA
L7	3	S L3 AND DETECT
L8	42	S L3 AND ACTIVITY
L9	6	S L8 AND PROTEASE
L10	0	S RENILLA AND LUCIFERASE AND CONSERVED
L11	0	S RENILLA AND LUCIFERASE AND CONSER?
L12	0	S RENILLLA AND LUCIFERASE
L13	369	S RENILLA AND LUCIFERASE
L14	5	S L13 AND PROTEASE
L15	5	DUP REM L14 (0 DUPLICATES REMOVED)
L16	63	S L13 AND REGION
L17	33	DUP REM L16 (30 DUPLICATES REMOVED)
L18	27	S L13 AND CLONING
L19	25	DUP REM L18 (2 DUPLICATES REMOVED)
L20	5	S L19 AND (SEA OR REINFORMIS)
L21	1	S REINFORMIS AND LUCIFERASE
L22	141	S RENIFORMIS AND LUCIFERASE
L23	0	S L22 AND CASPASE
L24	0	S L22 AND CONSERVED
L25	16	S L22 AND CLONING
L26	15	DUP REM L25 (1 DUPLICATE REMOVED)

FILE 'STNGUIDE' ENTERED AT 15:08:43 ON 01 APR 2002

L26 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 1998:221121 CAPLUS
DN 128:291113
TI Renilla **luciferase** and green fluorescent protein fusion genes
IN Szalay, Aldar A.; Wang, Gefu; Wang, Yubao
PA Loma Linda University, USA
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9814605	A1	19980409	WO 1997-US17162	19970924
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW				
	RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5976796	A	19991102	US 1996-771850	19961223
	AU 9745004	A1	19980424	AU 1997-45004	19970924
	AU 730040	B2	20010222		
	EP 934425	A1	19990811	EP 1997-943558	19970924
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2001501100	T2	20010130	JP 1998-516659	19970924
PRAI	US 1996-27657P	P	19961004		
	US 1996-771850	A	19961223		
	WO 1997-US17162	W	19970924		

AB A fusion gene is provided comprising the cDNA of Renilla **luciferase** and the cDNA of the "humanized" Aequorea green fluorescent protein. The "RG fusion gene" was constructed with Renilla cDNA linked at a modified 3' end to a 15-nucleotide linker sequence encoding Ala-Ala-Ala-Ala-Thr, followed by the 5' end of intact GFP cDNA; similarly, a second "GR fusion gene" was constructed with GFP cDNA linked to a 27-residue linker sequence encoding Gly-Try-Gln-Ile-Glu-Phe-Ser-Leu-Lys, followed by the 5' end of Renilla cDNA. The RG fusion gene produces a novel protein, the "Renilla-GFP fusion protein", which displayed both the **luciferase** activity of Renilla **luciferase**, and the green fluorescence of GFP, whereas the GR fusion gene product exhibited minimal response to UV light and demonstrated no energy transfer between the GFP and Renilla **luciferase** moieties. The Renilla-GFP fusion gene is useful as a double marker for monitoring gene expression quant. in UV light and by enzyme activity.

1: Gene 1999 Sep 3;237(1):153-9

Improved assay sensitivity of an engineered secreted Renilla luciferase.

Liu J, Escher A.

Center for Molecular Biology and Gene Therapy, Loma Linda University, CA, USA.

We have previously reported the construction of a functional Renilla luciferase enzyme secreted by mammalian cells when fused to the signal peptide of human interleukin-2. The presence of three predicted cysteine residues in the amino acid sequence of Renilla luciferase suggested that its secreted form could contain oxidized sulfhydryls, which might impair enzyme activity. In this work, four secreted Renilla luciferase mutants were constructed to investigate this possibility: three luciferase mutants in which a different cysteine residue was replaced by an alanine residue, and one luciferase mutant in which all three cysteine residues were replaced by alanine residues. Simian cells were transfected with the genes encoding these mutant luciferases, as well as with the original gene construct, and cell culture media were assayed for bioluminescence activity. Only media containing a mutated luciferase with a cysteine to alanine substitution at position 152 in the preprotein showed a marked increase in bioluminescence activity when compared to media containing the original secreted Renilla luciferase. This increase in light emission was due in part to enhanced stability of the mutant enzyme. This new enzyme represents a significant improvement in the sensitivity of the secreted Renilla luciferase assay for monitoring gene expression.

PMID: 10524246 [PubMed - indexed for MEDLINE]